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COMPOSITION AND FLAVOR MODIFICATION OF PROCESSED PEAS

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This report summarizes research carried on cooperatively by the National Canners Association and the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, between 1958 and 1964. The senior author, Jock W. Rolls, was employed by the Association and stationed at the Western Regional Research Laboratory, Albany, Calif., headquarters of the Western Utilization Research and Development Division. The coauthors are employees of the Division and the Division produced this report. Copies are available on request. Address Western Regional Research Laboratory, USDA, 800 Buchanan Street, Albany, California 94710

CONTENTS

	<u>Page</u>
INTRODUCTION - - - - -	5
COMPOSITION - - - - -	6
Nonvolatile Components - - - - -	6
Volatile Components - - - - -	9
Volatiles from the 1958 and 1959 Samples - - - - -	9
Volatiles from the 1960 Sample - - - - -	10
Volatiles Isolated from a Cannery Blancher - - - - -	14
FLAVOR STUDIES - - - - -	21
Flavor and Composition - - - - -	21
Additives - - - - -	22
Properties of Freeze-Dried Aqueous Extract - - - - -	24
Fractionation of Freeze-Dried Aqueous Extract - - - - -	26
Preparation of Samples - - - - -	27
Taste Panel Evaluation - - - - -	27
RELATED BASIC RESEARCH - - - - -	30
INDUSTRIAL APPLICATION - - - - -	31
SUMMARY - - - - -	33
ACKNOWLEDGMENTS - - - - -	34
LITERATURE CITED - - - - -	35
APPENDIX - - - - -	38
Isolation of Free Amino Acids from Freeze-Dried Aqueous Extract of Frozen Peas (FDAE) and from Frozen Peas - - -	38
Preparation of Volatile Fractions from 1960-Crop Peas and Preliminary Analysis by Gas Chromatography - - - -	38
Development of Procedure for Separating Volatile Components by Chemical Classes - - - - -	39
Analysis of Fraction 2 -- 1960 Peas - - - - -	41
Analysis of Fraction 3 -- 1960 Peas - - - - -	42
Artifacts of the Isolation Procedure -- 1960 Peas - - - -	44
Control Analyses and Tests -- 1962 Peas - - - - -	45
Preparation of Freeze-Dried Aqueous Extract (FDAE) - - -	47

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COMPOSITION AND FLAVOR MODIFICATION OF PROCESSED PEAS

JACK W. RALLS

National Cannery Association

Berkeley, California

and

JOHN F. CARSON, RICHARD M. SEIFERT and HORACE K. BURR

Western Utilization Research and Development Division,

Agricultural Research Service, U.S. Department of Agriculture, Albany, California

This report summarizes 6 years of research on a project directed toward improved canned pea flavor. In general, improvement of the flavor of preserved foods is desirable to encourage increased consumption of the more nutritious commodities. More specifically, improved flavor of the canned pea product would help to protect the substantial capital investment in the pea canning industry of the United States. The report is divided into four sections: (a) compositional studies, (b) flavor studies, (c) related basic research, and (d) industrial applications. These divisions reflect the research plan followed in the project.

We began by trying to discover the potentially flavorful substances that are present in peas. We believed these substances would be included in the following chemical classes: nonvolatile, including free amino acids, mono- and di-saccharides, and poly-functional carboxylic acids; and volatiles, including acids, aldehydes, alcohols, ketones, amines, and sulfur compounds. We discovered flavorful members of all these classes in peas. The very low concentration of volatiles in peas made it necessary to isolate them from large quantities and in some cases to develop new analytical techniques. The largest sample of pea steam volatiles was collected from a cannery blancher on activated charcoal. We analyzed this material with capillary-column gas chromatography and time-of-flight mass spectrometry.

When we had found in peas a considerable number of compounds that might contribute to flavor, we tried to learn whether they actually did so. Work on the relation of composition to flavor followed four research paths. The identified volatile components were analyzed for odor type according to the Amoore stereochemical theory of olfaction (1).¹ A second approach was determination of

¹Numbers in parenthesis refer to the bibliography on page 35.

the olfactory thresholds of individual pea components by a trained odor panel. A more substantial program correlated taste panel ranking of fresh, frozen, and canned pea samples with a series of analytical measurements. The fourth program was addition of identified flavorful components to experimental packs. The additives were evaluated by a taste panel. Those components that did not modify the flavor were dropped from further consideration. The compounds that improved flavor, according to the panel, were tested in various combinations in an attempt to develop an additive that would improve the flavor of canned peas.

Throughout the work it was often necessary to conduct research in fundamental organic chemistry to fill gaps in basic knowledge. Fundamental research was the third phase of the program. The areas investigated were: (a) nonenzymatic formation of acetoin, (b) reactions of lactams with diazoalkanes, (c) $O \rightarrow N$ thermal rearrangements, and (d) catalysis of thermal unimolecular rearrangements.

The industrial applications take the form of suggestions to pea processors on how to use the results to improve products. Research to improve varieties may be facilitated by objective measurements of one or more pea components. Any of several additives could be used in experimental packs, and the degree of improvement evaluated by quality control personnel. It is likely that experienced pea processors can integrate this new information with their own unpublished knowledge to improve flavor.

COMPOSITION

Nonvolatile Components

The composition of the green garden pea (Pisum sativum) has been studied in a number of laboratories around the world. A comprehensive review paper on pea composition is being written by one of the authors with Donald G. Crosby of the University of California, Davis. The review is one of Professor Crosby's series, "The Organic Constituents of Foods," and will be available in the Journal of Food Science. The present report discusses only the results obtained in the Western Utilization Research and Development Division, U.S. Department of Agriculture investigations. Our initial work on pea composition was done with samples from a pack of frozen Perfection peas prepared commercially in Oregon in 1958.

We studied the nonvolatile compounds first. The sugars and amino acids were extracted from the peas by homogenizing with absolute ethanol. They contained 3.99% total sugars, including 0.11% reducing sugars (calculated as glucose).² Paper chromatography

²All concentrations are reported on a wet-weight basis except the amino acids. The peas contained 17.4 percent total solids.

demonstrated that sucrose was the predominant sugar with traces of glucose, fructose, and raffinose.³ In a later paper-chromatographic study, sucrose was found to be the predominant sugar in pureed pea baby food, canned peas, and frozen peas. The minor sugars were glucose and fructose; traces of stachyose were present in the canned pea sample.⁴

At first we determined the free amino acids by two-dimensional paper chromatography. The solvent system in the first direction was phenol-water and that in the second direction, n-butanol-acetic acid-water. The predominant amino acids measured after spraying the chromatograms with ninhydrin solution were glutamic acid, methionine, threonine, homoserine, and alanine. More recent quantitative determinations of free amino acids in peas have been made on an automatic amino acid analyzer.

Free amino acids in frozen peas and in the freeze-dried aqueous extract of frozen peas (FDAE) were determined by the general procedure described in the Phoenix Precision Instrument Company Manual for tissue extracts. The manual is based on a paper by Stein and Moore (22). Isolation procedures are given on page 38. (The FDAE from frozen peas represents about a 15-fold concentration of the fresh weight of frozen peas, or a 3-fold concentration on a dry-weight basis, see page 47.)

Table 1 lists the amino acids in micromoles per milligram dry weight of frozen peas, or of FDAE, for hydrolyzed and non-hydrolyzed samples. Acetoin and its companion compound, biacetyl, had been identified in peas by previous workers (5). A colorimetric method for the determination of acetoin was adapted from that of Westerfeld (26). The amount of acetoin in frozen peas varied with the duration of heating at 100°C.⁵ It increased rapidly from 26 to 34 p.p.m. in 15 minutes and then fell to 28 p.p.m. within 30 minutes. The reason for this increase was investigated, and the results are discussed on page 30.

Another class of nonvolatile compounds measured was the poly-functional organic acids. The three predominant nonvolatile acids, citric, malic, and pyrrolidone carboxylic acids, were determined (table 2) by a method combining ion-exchange paper chromatography and titration (9). The less abundant acids were determined by

³Unpublished data, K. T. Williams, formerly of this Division, July 3 and September 9, 1958.

⁴Unpublished data, A. L. Potter of this Division, June 12, 1962.

⁵All temperatures are centigrade.

TABLE 1.--Amino acid composition of frozen peas and of the
freeze-dried extract (FDAE) of frozen peas
[μmoles/mg. (dry weight)]

Amino acid	FDAE, not hydrolyzed	FDAE, hydrolyzed	Frozen, not hydrolyzed	Frozen, hydrolyzed
As leucine				
¹ X ₁	0.002	--	0.001	--
X ₂	.083	.001	.036	--
X ₃	.001	--	trace	--
X ₄	.01	--	.005	--
X ₅	.002	.001	.001	.001
X ₆	.0008	.001	.0004	.0004
X ₇ leucine	.008	.009	.004	.004
Asparagine	.009	.04	.002	.013
Threonine	.082	.07	.03	.026
Serine	² .029	.02	.009	.007
Glutamine	.099	.18	.04	.084
Proline	.01	.013	.003	.006
Glycine	.01	.025	.005	.009
Alanine	.19	.19	.099	.099
1/2 Cystine	.002	.003	trace	trace
Valine	.032	.035	.014	.015
Methionine	.002	.002	.001	.001
Isoleucine	.014	.015	.007	.007
Leucine	.007	.008	.003	.004
Tyrosine	.004	.001	.002	.001
Phenylalanine	.004	.004	.002	.002
Homoserine	.027	.008	.01	.002
α-Amino butyric	.005	.006	.003	.003
Lysine	.03	.02	.015	.01
Histidine	.008	.007	.004	.004
Arginine	.20	.22	.11	.12
Canavanine	.01	trace	.006	trace

¹X indicates unknown compounds.

²Estimate.

treating ammonium carbonate eluates from an ion exchange column with diazoethane and measuring the resultant ethyl esters by gas chromatography. In this way, lactic, pyruvic, acetoacetic, and oxalic acids were determined (table 2).

Some information on the fat content was obtained by extracting with hexane the lipids from 150 pounds of freeze-dried peas. Evaporation of the hexane left an orange-red oil (172 g., 0.25 percent). Adsorption chromatography of the oil on alumina-Celite according to the method of Huang et al. (8) provided some β-sitosterol and a triglyceride fraction accounting for 0.10 percent of

the weight of the peas. The fatty acid composition was determined by base-catalyzed exchange with methanol and gas chromatography of the resulting methyl esters. The triglyceride fatty acids had the following abundance: linoleic (51%), palmitic (21%), oleic (17%), linolenic (5%), stearic (3%), myristic (2%), and arachidic (0.5%).

TABLE 2.--Some flavorful nonvolatile components of peas

Component	P.p.m. of fresh wt.	Component	P.p.m. of fresh wt.
Sucrose	38,800	Malic acid	520
Glucose } Fructose }	980	Pyrrolidone carboxylic acid	380
Raffinose	trace	Lactic acid	170
Stachyose	trace	Pyruvic acid	150
Acetoin	33	Acetoacetic acid	80
Citric acid	960	Oxalic acid	20

Volatile Components

The volatile components of peas have received careful attention because many research workers believe that the odor of food is more important than the taste in establishing flavor. We concentrated first on the carbonyl compounds and volatile acids. We soon found that a new and more sensitive method for measuring these compounds in dilute water solution was required. We developed the method of flash exchange gas chromatography (13), a technique which requires only a few milligrams of the compounds. Volatile aldehydes, ketones, acids, and mercaptans (3) are isolated as solid derivatives, regenerated in rapid exchange reactions, and volatilized directly into a gas-liquid partition chromatography (GLPC) unit for separation and identification. Aldehydes and ketones are determined by regeneration from their 2,4-dinitrophenylhydrazones (2,4-DNPH) with α -ketoglutaric acid. Monobasic carboxylic acids are transformed from their potassium salts into ethyl esters by heating with potassium ethylsulfate. Mercaptans are regenerated from mercuric mercaptides with toluene-3,4-dithiol.

Volatiles from the 1958 and 1959 Samples

The volatile carbonyl compounds (14) from the pea steam distillates are tabulated in tables 3 and 4. Duplicate determinations of acids in the steam distillate of 1959 crop peas gave 0.33 and 0.46 mg. formic acid per kg. peas, 0.024 and 0.029 mg. acetic acid, and 0.067 and 0.060 mg. iso-valeric acid. Flash exchange gas chromatography was used to determine aldehydes, ketones, and acids. The biacetyl was measured by the modified Westerfeld colorimetric method. All the components occurred at less than 3 p.p.m. A mixture of these aldehydes, ketones, and acids at the concentration levels found did not reproduce the characteristic odor of pea steam distillate.

TABLE 3.--Concentration of volatile carbonyl compounds
in initial steam distillate of peas

Compound	Average concentration in peas, mg./kg.	
	1958	1959
Acetaldehyde	2.4	0.56
Acetone	0.35	.12
Propionaldehyde	.17	.00
<u>n</u> -Butyraldehyde	.13	.06
<u>n</u> -Valeraldehyde	.00	.44

TABLE 4.--Change in biacetyl composition of steam distillate
of peas (1958) with time of distillation

Distillation time	Volume distillate	Weight 2,4-DNPH ¹	Weight due to biacetyl	Biacetyl content of peas
(Min.)	(Ml.)	(Mg.)	(Mg.)	(Mg./kg.)
33	275	15.1	6.27	0.61
57	263	5.05	4.04	.39
81	310	3.59	3.58	.35
99	258	2.96	3.02	.29

¹2,4-Dinitrophenylhydrazone.

Two thousand pounds of frozen peas of the 1959 crop were steam distilled and non-polar compounds were extracted from the foam distillate with a commercial hydrocarbon solvent. When the solvent was distilled off, the remaining oil contained nearly as much material (400 mg.) from solvent residue as from the peas (630 mg.). This experience emphasized the importance of using rigorously purified solvents and larger quantities of peas.

Volatiles from the 1960 Sample

Volatiles were extracted from 5000 pounds of frozen peas with highly purified iso-pentane. The extract was then separated into 3 fractions by distillation and each of these was analyzed by gas-liquid partition chromatography (GLPC) (figs. 1, 2, 3). Details of this work are given on pages 38, 39.

Because GLPC showed the pea extract concentrate to be very complex, we used an isolation scheme (see pages 39-41) which separated the mixture into chemical classes in order to facilitate identification of components by GLPC and mass spectrometry.

The extract was separated into chemical classes as indicated in figures 4 and 5. The less abundant fractions were tentatively identified by GLPC retention times on three different substrates as compared to reference compounds (see pages 41-44).

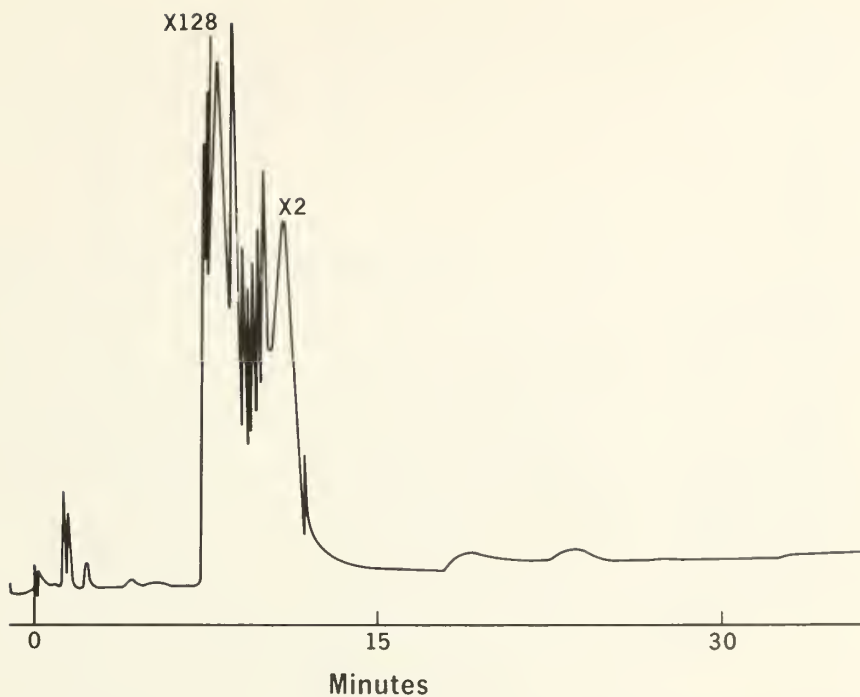


Figure 1.--Gas chromatogram of fraction 1, 1960-crop peas.
Column temperature 50°C.

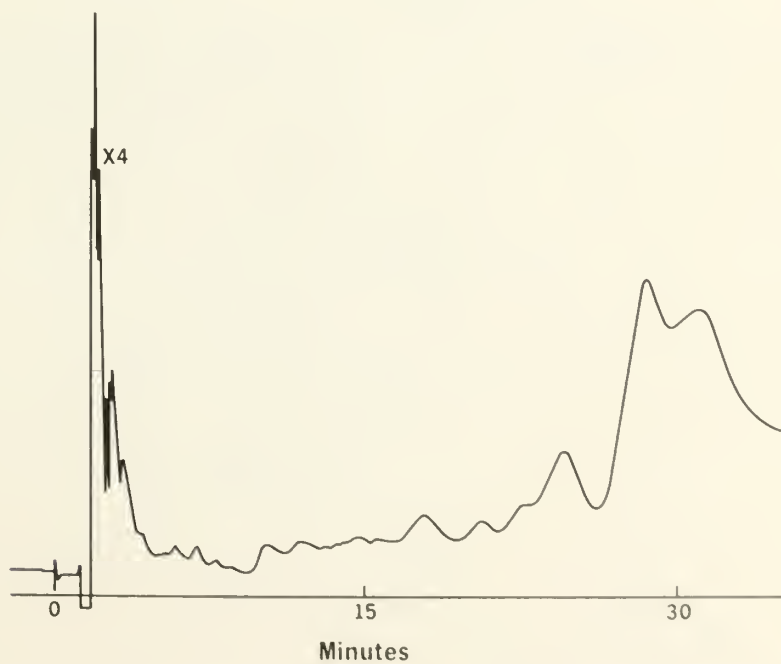


Figure 2.--Gas chromatogram of fraction 2, 1960-crop peas.
Column temperature 153°C.

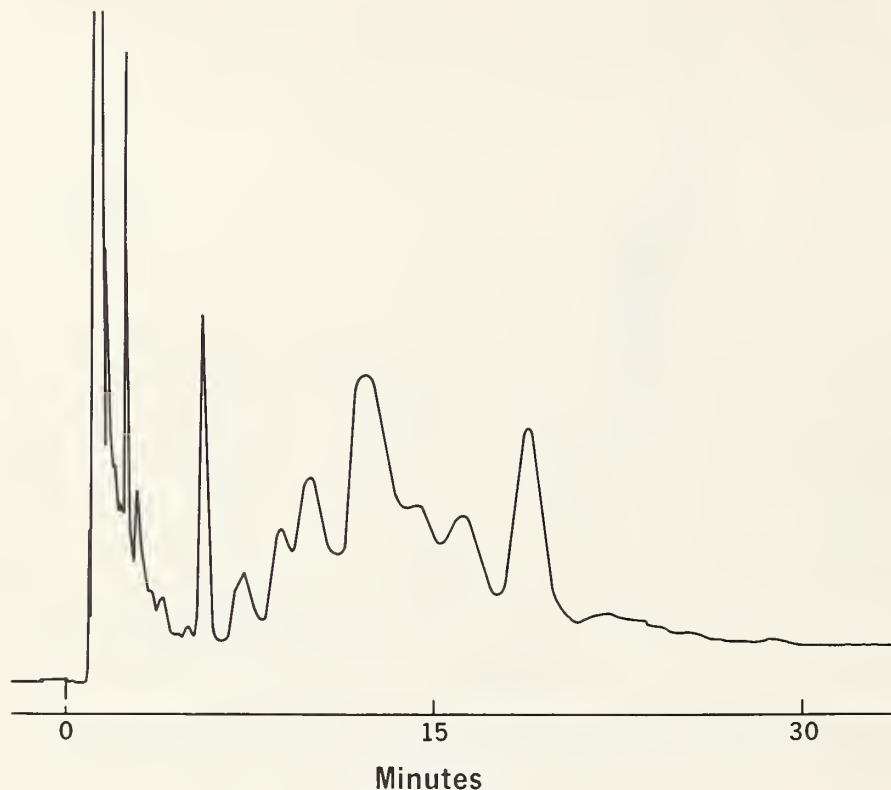


Figure 3.--Gas chromatogram of fraction 3, 1960-crop peas.
Column temperature 205°C.

The most abundant portion of fraction 2 was analyzed on a capillary column gas chromatograph coupled to a time-of-flight mass spectrometer, the "Cap-MS technique" (10). As each component was eluted from the column, the mass spectrum was recorded in about 1 to 4 seconds. This method made it possible to discover many classes of compounds (page 13) but often failed to identify a compound exactly. For instance, the technique indicated the presence of a large number of aliphatic saturated hydrocarbons containing 8 to 12 carbon atoms. Unfortunately, the mass spectra of many of these compounds are very similar.⁶ The molecular ions are always in low abundance or even nonexistent if the structure contains a moderate degree of branching. Thus, even the molecular weight may be uncertain. A great many similar hydrocarbons were eluted in about 70 minutes. Full separation and identification were impossible because the mass spectrograph had to scan each outcoming spectrum so rapidly. The large number of hydrocarbon spectra with similar retention times probably represents the large number of isomers of C_8 - C_{12} aliphatic hydrocarbons that are possible. The number of isomers in this group that are

⁶Private communication, W. H. McFadden of this Division.

known to exist is 642. Alternatively they may represent continuous evolution of hydrocarbons in the stream of gas inside the capillary column. Such evolution could result from decomposition of some class(es) of compounds (possibly sulfides) on the stainless steel walls.

Steam volatile components of peas -- 1960 crop

Identified by GLPC retention times

formic acid	hexanoic acid	<u>n</u> -heptyl alcohol
acetic acid	lauric acid	<u>sec</u> .-decyl alcohol
propionic acid	palmitic acid	<u>iso</u> -valeraldehyde
<u>n</u> -butyric acid	<u>sec</u> .-undecyl alcohol	
<u>iso</u> -valeric acid	stearic acid	

Esterified alcohols

<u>iso</u> -amyl alcohol	<u>iso</u> -heptyl alcohol	<u>n</u> -heptyl alcohol
<u>n</u> -amyl alcohol	<u>n</u> -nonyl alcohol	<u>iso</u> -octyl alcohol
<u>sec</u> .-hexyl alcohol	<u>sec</u> .-nonyl alcohol	<u>sec</u> .-decyl alcohol
<u>n</u> -heptyl alcohol	<u>n</u> -octyl alcohol	<u>iso</u> -nonyl alcohol
<u>sec</u> .-heptyl alcohol	<u>n</u> -undecyl alcohol	<u>n</u> -decyl alcohol
<u>sec</u> .-octyl alcohol		

Identified by GLPC-mass spectrometry

ethylbenzene	methylpropylbenzenes
<u>o</u> -dimethylbenzene	dimethylethylbenzenes
<u>m</u> -dimethylbenzene	diethylbenzenes
<u>p</u> -dimethylbenzene	tetramethylbenzenes
methylethylbenzenes	limonene
trimethylbenzenes	α -terpinene (tentative)
isopropylbenzenes	dimethyl disulfide
possible pyran or methyl furan	indene (tentative)
possible methyl-indane or <u>p</u> ,	indane (tentative)
α -dimethylstyrene	

Identified by infrared spectrum and by combustion sulfur

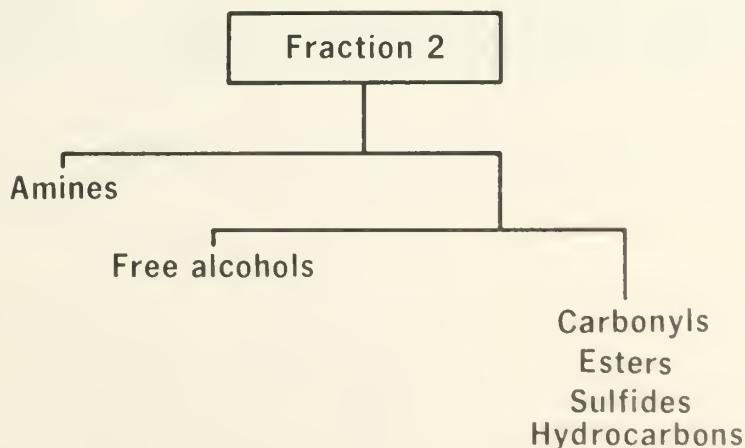


Figure 4.--Separation of fraction 2 into chemical classes.

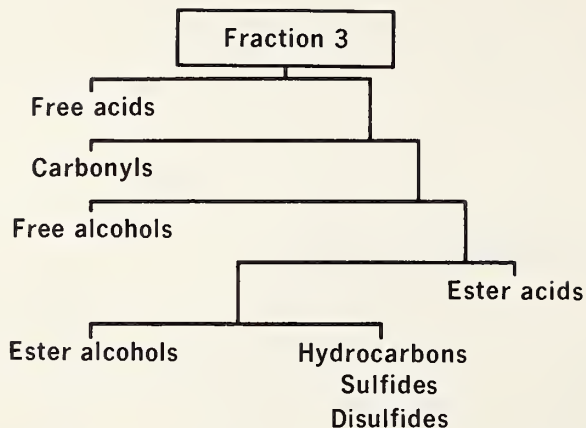


Figure 5.--Separation of fraction 3 into chemical classes.

The spectra of aliphatic ketones are similar to those of the saturated hydrocarbons. Under the rapid-scan conditions of the present experiments, it was not always possible to distinguish between them.

The volatiles included two $C_{10}H_{10}$ terpene hydrocarbons. Limonene, which has a characteristic mass spectrum, was present in considerable amounts. The other terpene was only a trace; the spectrum was characteristic of α -terpinene and its chromatographic elution time was consistent with α -terpinene.

Volatiles Isolated from a Cannery Blancher

The isolation of volatile components in experiments using 2,000 and 5,000 pounds of peas gave so little concentrate that artifacts of isolation confused the results. The most logical source of volatile material from large quantities of peas is a commercial blancher.

This section of the report discusses material isolated from a steam blancher in a California pea cannery during the 1962 season. The fractionation procedure was that shown in figures 4 and 5. The compounds isolated were identified primarily by the Cap-MS technique of McFadden and Teranishi (private communication).

The volatiles from a steam blancher were collected with the equipment shown in figure 6. The collector was installed on a roof adjacent to a commercial steam blancher, and the steam from hot Perfection peas pumped out of the blancher by a Nash pump. It passed through a 3-inch pipe to a water-cooled condenser. Vapor from above this condensate was further pumped through a 1-inch-diameter pipe filled with activated charcoal (Columbia AC, 8-10 mesh). The collection equipment was operated continuously for

17 days except when the blancher was washed out at the end of each shift. Two hundred gallons of condensate was collected. Twenty-four pounds of charcoal was exposed to the condensate vapor in 12 separate 2-pound portions which were changed every 24 or 48 hours. The wet charcoal smelled like peas.

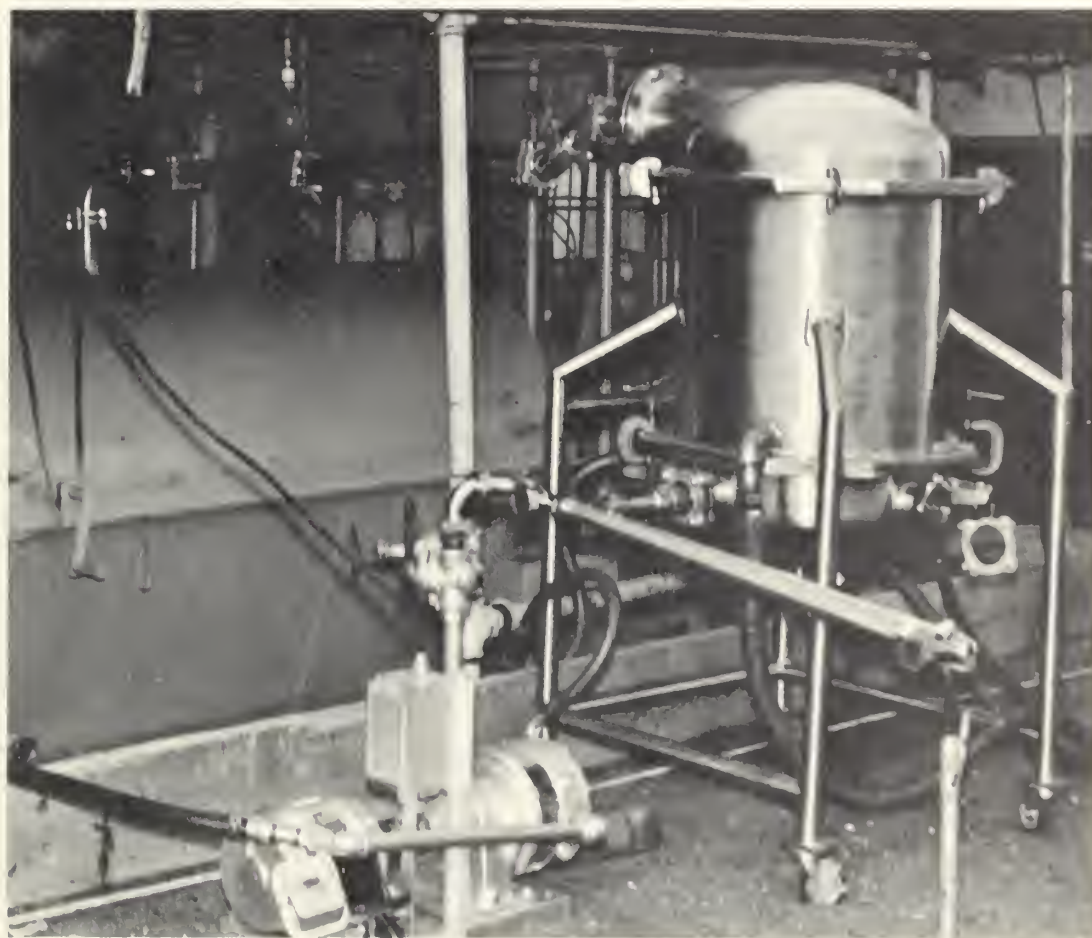


Figure 6.--Equipment used for collecting pea volatiles from a steam blancher, 1962 season, showing blancher (left), condenser (right), horizontal, U-shaped charcoal tube (right center), and vacuum pump (bottom).

The odor on the charcoal largely remained after removal of water by freeze-drying. Separate portions of the dried charcoal were washed with four solvents, ethyl ether, methanol, ethyl acetate, and dichloromethane. The extracts gave complex GLPC spectra. Identical washing of unexposed (fresh) charcoal gave much less complicated GLPC recordings. The remainder of the

exposed dried charcoal (20 pounds) was washed (Soxhlet extractors) with 6 liters of purified dichloromethane (distilled through a 25-plate Oldershaw fractionating column, boiling point 40.3°-40.5°C.).

The dichloromethane extract of the charcoal was concentrated by distillation through a fractionating column at atmospheric pressure. The residual liquid (about 150 ml.) was distilled at reduced pressure. The 94.5 ml. of collected distillate was redistilled to give 42.6 g. of liquid, b.p. 70°-78°/754 torr, n_D^{25} 1.3636. This material was purified by preparative GLPC and identified as ethanol by its infrared spectrum and by preparation of two derivatives (ethyl 3,5-dinitrobenzoate, m.p. 90.5°-91.5°C.; and ethyl α -naphthyl urethane, m.p. 77.5°-78°C.).

After removal of the ethanol, the residue separated into two phases. The upper layer had a volume of 33 ml. and the lower phase, 24 ml.

The major component of the lower phase of the concentrated extract was isolated by GLPC fractionation and identified as dimethylsulfoxide (DMSO) by carbon-, hydrogen-, and sulfur-analysis, infrared spectra, mass spectra, and oxidation to dimethyl sulfone, m.p. 109.5°-109.9°C. The rest of the lower phase was separated into four crude fractions by GLPC on a 6-foot by 1/4-inch 10 percent Carbowax 1540 column at 105°C. and a helium flow rate of 45 ml./minute.

The upper layer (nonpolar phase) of the concentrated extract was also separated into 4 fractions on a 20-foot by 3/8-inch 25 percent Carbowax on Chromosorb P column at 108°C. with a helium flow rate of 170 ml./minute (see fig. 7).

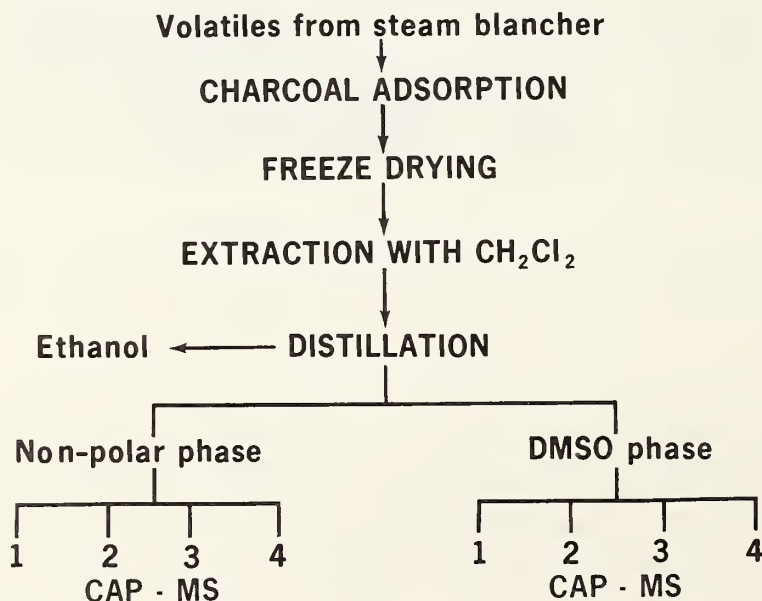


Figure 7.--Recovery and separation of pea volatiles.

The individual fractions from the nonpolar and DMSO phases were qualitatively analyzed by the Cap-MS technique. The non-polar phase fractions were analyzed on a 250-foot by 0.01-inch capillary column coated with Carbowax 1540.

Effluent mass spectra were monitored by visual display of the pattern on an oscilloscope and a GLPC trace was obtained simultaneously by recording the ionization due to mass 43 ($C_3H_7^+$, CH_3CO^+ , etc.). Because the percentage of ionization due to a particular mass varies from one material to another, chromatograms obtained by this method are completely nonquantitative but nevertheless do provide a useful record for general reference.

For example, figure 8 shows the chromatogram thus obtained during a temperature-programmed separation of the DMSO-fraction 1. Figure 9 shows representative mass spectra during the elution of peaks in the temperature range 105°-110°C. At the top is a small section of the chromatogram shown in figure 8. This indicates the places the mass spectra were taken.

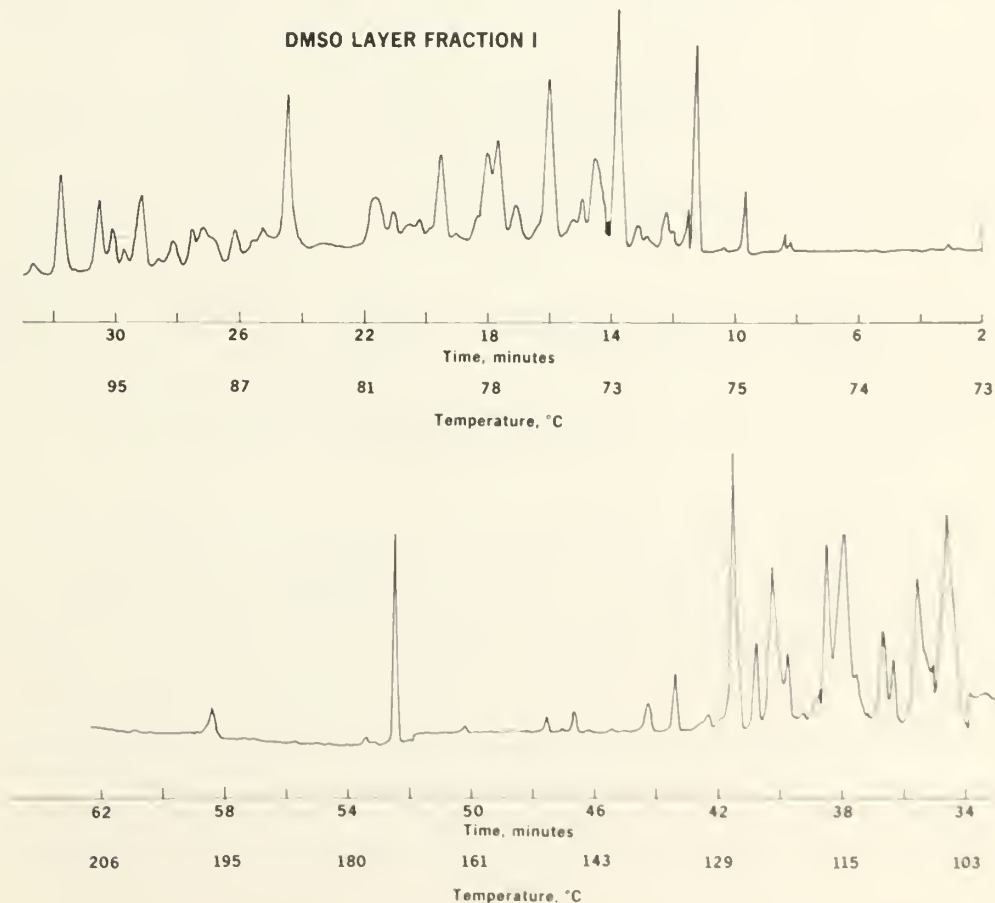


Figure 8.--Gas chromatogram of DMSO -- fraction 1.

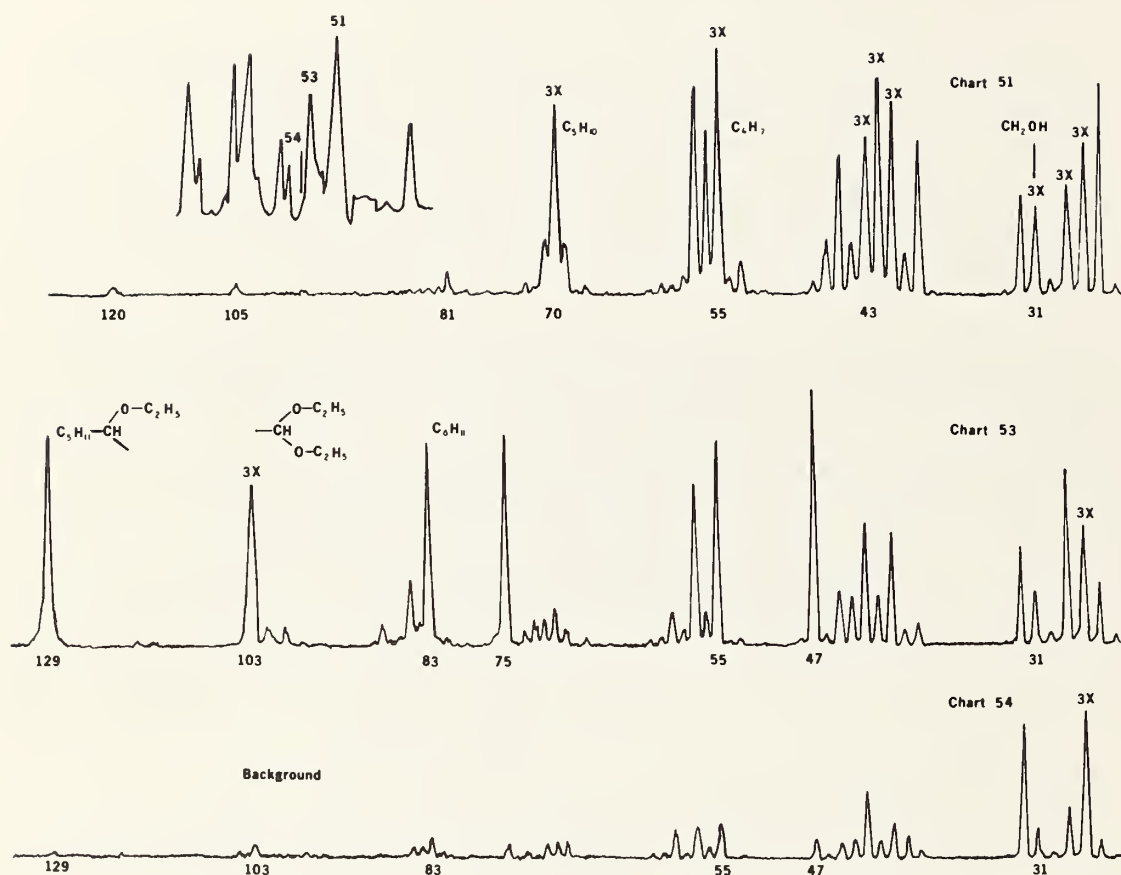


Figure 9.--Representative mass spectra obtained with "Cap-MS" procedure.

Mass spectral identification was made with available reference spectra or by comparing the mass spectra from authentic compounds. Whenever possible, the retention times of the tentatively identified components were determined on the same column with a hydrogen flame ionization detector.

The possibility of artifacts of isolation is always a serious problem in analysis of trace components (see pages 45, 46). To estimate the mass and composition of the impurities, fresh dichloromethane and charcoal from our stocks were run as blanks and analyzed. Interpretation of the mass spectra obtained from the analysis of the charcoal-dichloromethane blank is summarized below. The material from the impurities on the charcoal or in the solvent would have weighed a calculated maximum of 25.92 g.

Major impurities in the solvent-charcoal blank:

<u>Halogenated:</u>	<u>Aromatic hydrocarbons:</u>
1,2-dichloroethane	benzene
tetrachloroethene	toluene
bromobenzene	naphthalene
	6 indans and indenenes
<u>Aliphatic hydrocarbons:</u>	27 alkylated benzenes
16 C ₈ -C ₁₅ saturated and unsaturated	

Most of the major components identified in the blank run were not found in the exposed charcoal. Any components found in both the control and exposed charcoal are not reported as components of the pea volatiles.

Table 5 summarizes the identity of the components in the various concentrate fractions. The charcoal extract concentrate weighed 118 g. The major component of this mixture was ethanol. Ethanol has been identified in peas by a number of previous investigators (5, 25).

The nonpolar fractions were composed principally of saturated hydrocarbons in the C₁₀ to C₁₄ range. The excessively broad eluted peaks and the mass spectral patterns showed that many isomers were present. A few unsaturated C₁₀ to C₁₄ hydrocarbons were observed. Since these hydrocarbons could be present in the steam entering the blancher they are not listed in table 5.

As was pointed out, the chromatograms were obtained by recording the ionization at mass 43 and do not measure the components. The entries in table 5 are designated 5, 4, 3, 2, and 1 (very strong, strong, moderate, weak, very weak) on the basis of the recorded mass spectral ionization.

Mass spectral evidence alone cannot always be relied upon to give positive identification, and even with supporting gas chromatographic evidence the results should be regarded carefully. The entries in table 5 are all fairly well established. Not listed are quite a few compounds (generally 1 to 3) whose mass spectra only suggested structures. These include a few saturated and unsaturated esters, alcohols, and acetals, a few additional aldehydes, and possibly some alkyl furans and other heterocyclic structures. However, because the mass spectra of furans can be confused with those of conjugated dienals or of some diunsaturated hydrocarbons, further work will be required for certain identification.

The component eluted at 52 minutes from the DMSO fraction 1 (fig. 8) is of considerable interest because it does not seem to be in the general classes of other compounds found. Because the

most abundant mass spectral peak was mass 43, the chromatographic trace is strong relative to the total mass spectral pattern. The molecular weight is apparently 132 (the largest mass peak observed). There is no indication of aromaticity of hydrocarbon chains greater than 3 or 4 carbons. Mass peaks observed at 47, 48, and 49 were weak, but indicated a possible sulfur compound. However, the various propylbutyl sulfides (t-butyl not tested) did not match the observed spectrum. Isobutyl and n-butyl thioacetate were also synthesized but did not conform. Further research will be necessary in order to identify this compound.

TABLE 5.--Volatile compounds of steam from
blanching of green peas

Abundance level ¹	
<u>Alcohols:</u>	<u>Aldehydes:</u>
methanol 3	ethanal 4
ethanol excess	butanal 3
1-propanol 5	2-methylbutanal 4
2-methyl-1-propanol 3	3-methylbutanal 4
1-butanol 2	(<u>iso</u> -valeraldehyde)
1-pentanol 3	pentanal 5
2-methyl-1-butanol 4	hexanal 4
3-methyl-1-butanol 3	
1-hexanol 5	<u>Esters:</u>
3-hexene-1-ol 5	ethyl formate 5
a heptanol 3	ethyl acetate 5
(primary, branched)	ethyl propanoate 2
	ethyl pentanoate 2
<u>Acetals</u> ²	methyl octanoate 4
diethoxy methane 3	(branched)
diethoxy ethane 3	ethyl lactate 5
diethoxy butane 3	hexenyl acetate 3
diethoxy pentane 4	hexyl acetate 4
(presumably 2-Me-butane and 3-Me-butane)	
diethoxy <u>n</u> -pentane 4	<u>Sulfur compounds:</u>
diethoxy hexane 5	dimethyl sulphide 4
ethoxy pentoxy butane 2	dimethyl disulphide 4
ethoxy pentoxy pentane 3	dimethyl trisulphide 3
ethoxy propoxy hexane 1	carbon disulphide 1
ethoxy pentoxy hexane 1	methyl mercaptan 1

¹The values 5, 4, 3, 2, 1 stand for very strong, strong, moderate, weak, and very weak, respectively. The factor of 3-5 separates levels so that level 5 is about 300 times level 1.

²May be artifacts of isolation procedure.

Artifacts produced by reactions of components originally present in peas are of considerable concern. DMSO definitely arises from catalytic autoxidation of dimethyl sulfide. There was no DMSO in the condensate collected directly from the blancher. A model experiment demonstrated that DMSO formed by oxidation of dimethyl sulfide adsorbed on the charcoal. Dimethyl sulfide was the second most abundant organic compound of the pea blancher steam.

Identification of aldehyde diethyl acetals in the charcoal extract concentrate is novel and exciting. We tried to establish by runs under model conditions if these acetals were originally present in the peas or formed through reaction of the aldehydes and ethanol adsorbed on the charcoal. The exact catalytic conditions existing on the charcoal in the large scale isolation would be very difficult to duplicate. The total composition of the absorbate could influence the position of equilibrium in the formation of acetals. The contribution of acidic components in the mixture to the total catalytic effect would be especially important. Some acetal formed under model conditions; however, the proportion of acetal to the parent aldehyde was much smaller than that in the pea extract concentrate. The model is necessarily imperfect. The disproportion indicates some of the acetals come from the peas, but additional evidence is required before it can be stated that acetals are true components of the vapor above heated peas.

FLAVOR STUDIES

Flavor and Composition

The first comprehensive attempt to find out how composition affects flavor had the following experimental plan. Samples of commercial fresh, frozen, and canned peas were ranked by the Food Appraisal Investigations panel for best pea flavor. Samples of the peas were analyzed and the composition correlated with the flavor ranking.

Experimental samples for the above sensory and chemical measurements were prepared as follows: Commercial canned peas were placed in a 10-gallon, steam-jacketed kettle and heated to 90°C. during 7 minutes. The hot peas were ladled into the delivery tray of a Fitzpatrick mill (0.0465-inch mesh screen) and ground. The puree was stirred to uniform consistency and placed in C-enamel-lined, 303 x 406 cans and the cans hot closed. After the cans had been washed and dried, the samples were frozen in a blast freezer. The operation lasted 55 minutes. After 20 hours in the blast freezer the cans were stored in a -20°F. cold room.

Fresh market peas (grown at Santa Paula, California) were mechanically podded. Thirty-six pounds of cleaned seeds were added to 10 quarts of boiling water which contained 252 g. of salt. The peas were cooked at a rolling boil for 10 minutes. The cooked fresh peas were ground, canned, frozen, and stored as described for the canned pea sample above.

Fifty-eight 10-ounce packages of frozen peas were cooked in 9 quarts of water containing 208 g. of salt. After cooking for 5 minutes at a rolling boil, the peas were ground, canned, frozen, and stored as described above for the canned pea sample.

A taste panel ranked the flavor of the three pea samples in descending order: fresh, frozen, and canned.

An aromagram of each sample was taken on a chromatograph with 1000-foot x 0.034-inch i.d. nylon capillary columns (coated with GE Silicone 96) and a dual hydrogen flame detector. The columns were operated at 40.5°C. with a nitrogen carrier gas pressure of 5.7 p.s.i.g. The hydrogen pressure on the second stage of the regulator was 9 p.s.i.g.

Hundred-gram samples of pea material were diluted with 50 ml. of water. The suspension was stirred with a Teflon-coated magnetic stirring bar and heated on a hot plate until the internal temperature was 93°-95°C. The sample was removed from the hot plate, and the tip of a 3-ml. syringe was held 1/2-inch above liquid level. When the internal temperature reached 90°C., 2 ml. of vapor was pulled into the syringe. A 3/4-inch-long, 25-gauge needle was placed on the syringe, and the vapor sample was injected into the chromatograph.

The aromagrams of the three pea samples and of a control are shown in figure 10. The results of the selected analytical measurements on the three types of pea samples are tabulated in table 6. There was no significant correlation between the data from the objective measurements given in table 6 and the taste panel ranking.

Additives

A freeze-dried aqueous extract (FDAE) was prepared from frozen peas and tested as a flavor adjunct and as an additive incorporation agent in canned peas. The preparation of the FDAE is described on page 47. The extract gave canned peas an odor similar to that of cooked frozen peas, but the two aromas were readily distinguishable. Addition of the extract to canned peas up to 1 to 2 percent did not improve flavor. However, the FDAE material did demonstrate the presence in peas of several components unreported by Pendlington and Ward (11). The composition

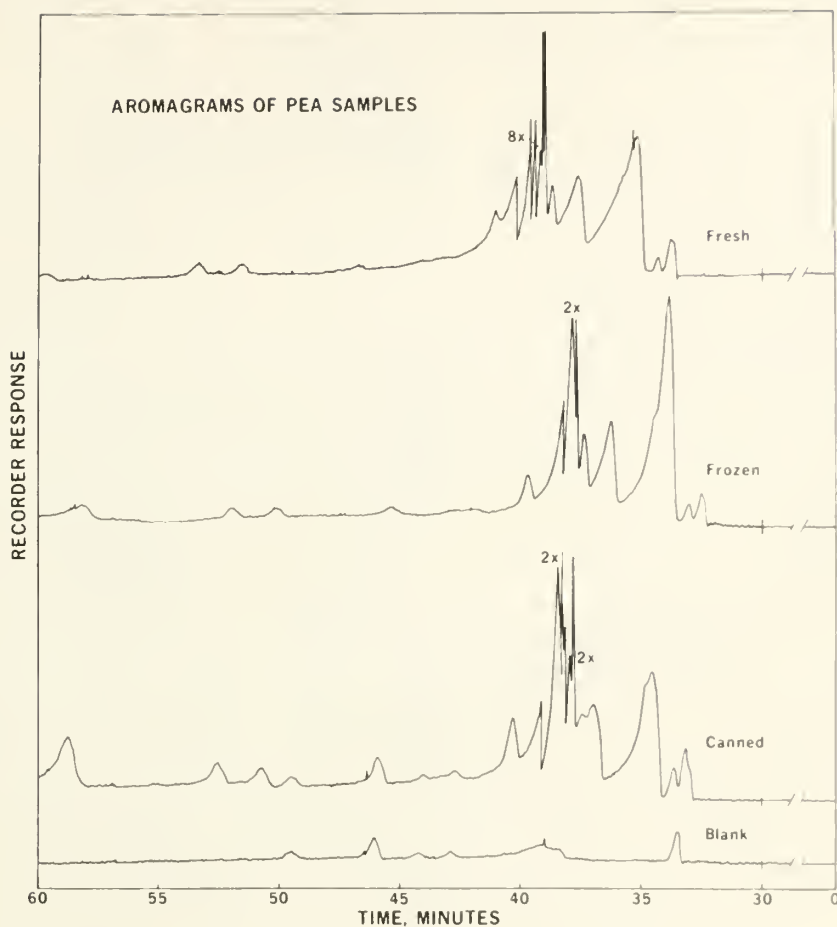


Figure 10.--Aromagrams of fresh, frozen, and canned pea samples.

of the extract powder served as a starting point for selection of additive substances to modify canned pea flavor in experimental packs. Sensory testing of canned pea samples eliminated several of those substances. The in situ generation of dimethyl sulfide in canned peas by a mixture of pectin and methionine gave the highest panel acceptance of several additives tested.

Since best flavor probably results from a critical balance of contributing components, portions of the complex freeze-dried pea extract were used as additives in canned pea samples and their contribution to flavor evaluated.

The high phospholipid content of the FDAE suggested it might also be surface active. The material might make the intact frozen peas more permeable and increase their uptake of other additives.

This function of the FDAE was tested by adding 0.1 percent of the extract powder (based on the final weight of canned peas) to a four-component additive mixture. The presence of FDAE did not make the additives we tried more effective.

TABLE 6.--Selected analyses of pea samples

Analyses	Fresh	Frozen	Canned
Steam volatile carbonyls, mg. 2,4-DNPH/500 g.	25.8	22.1	21.3
Carbonyls, peak area sq. mm./5 mg. 2,4-DNPH			
acetaldehyde	562	632	396
propionaldehyde	0	0	67
acetone	267	147	121
<u>iso</u> -butyraldehyde	20	12	8
pentanal	45	23	19
hexanal	0	0	6
AlS, percent	7.34	7.17	6.77
Ash, percent	1.41	1.39	0.96
Reducing sugars, percent	.40	.58	.77
Biacetyl + acetoin, µg./g.	33	16	34
Acids, peak area/5 mg. K salt			
formic acid	597	381	132
acetic acid	700	730	1,050
<u>iso</u> -valeric acid	690	350	912
Volatile mercaptans, p.p.m. as EtSH	0.42	0.72	0.39
Ammonia, p.p.m.	48.7	38.9	22.4
Amino nitrogen, p.p.m.	1,250	1,250	615

Properties of Freeze-Dried Aqueous Extract

Aromagrams were made of blended frozen peas, the freeze-dried aqueous extract (FDAE) of frozen peas, and a distilled water blank. An equivalent amount of each sample, 15 g. on a dry weight basis, in 150 ml. of distilled water was placed in a 250-ml. Erlenmeyer flask fitted with a thermometer well and thermometer. A Teflon-covered magnetic stirrer was dropped into the Erlenmeyer, and the top covered with aluminum foil. The flask was heated with stirring until the temperature reached 95°C. (about 12 minutes). A 5-ml. syringe without needle was used to take a 5-ml. vapor sample (25 seconds' exposure to vapor). A 1-inch, 23-gauge needle was quickly fitted to the syringe, and the sample was rapidly injected into a 1/8-inch x 10-foot column packed with 10 percent silicone SF 9600 on 60-80 mesh Chromosorb and maintained at 70°C. The carrier gas, nitrogen, was at 10 p.s.i.g., and the detector was a hydrogen flame type. The recorder was a Varian G-14, of 1 mv. full-scale sensitivity. The chart speed was 40 inches/hour.

TABLE 7.--Peaks from aromagrams of peas and pea extract

Peak No.	Elution time	Relative peak areas from vapor chromatograms		
		FDAE of frozen peas	Frozen peas	Water blank
	Minutes			
1	0.59	--	--	small
2	.65	--	--	small
3	.71	small	1.06	
4	.78	small	--	small
5	.83	--	small	--
6	.86	2.12	--	0.88
7	.89	270	small	
8	.94	--	--	small
9	1.00	--	4.70	
10	1.30	200	1,170	129
11	1.59	18.8	--	--
12	1.95	--	1.94	--
13	2.30	small	--	small
14	2.87	small	--	--
15	2.95	37.7	3.53	.23
16	3.60	13.2	34.1	.29
17	4.13	small	--	--
18	4.77	4.95	5.30	small ¹
19	5.55	0.41	10.7	small ¹
20	6.30	7.75	2.3	--
21	7.55	100	100	
22	8.60	0.18	.35	--
23	9.35	--	0.23	--
24	10.0	--	1.76	--
25	10.5	2.72	--	--
26	11.6	0.17	.59	--
27	13.5	--	.47	--
28	15.4	1.18	.41	--
29	16.9	2.65	5.18	--
30	19.5	.47	27.6	--
31	23.4	1.88	4.92	--
32	26.2	14.8	5.4	--
33	32.0	--	.09	--
34	34.0	4.42	.09	--
35	39.0	2.24	--	--
36	44.2	--	6.78	--
37	51.9	--	.59	--
38	54.7	2.65	7.72	--
39	59.7	2.35	--	--

¹Plateau formed by peaks.

Relative peak areas from the aromagrams (table 7) show that 18 of the measured peaks coincide.

Fractionation of Freeze-Dried Aqueous Extract

Fractions were extracted from the FDAE in separate experiments with ethanol, 1:1 ethanol-water, ethyl ether, and benzene. After evaporation of the solvent, each residue was simmered in water and the aroma evaluated. The results indicated that the key components were very soluble in water and that nonpolar solvents extracted some aroma-producing compounds uncharacteristic of peas.

A solution of 5 g. FDAE in 50 ml. of water centrifuged at 40,000 r.p.m. (average 105,400 x g.) for 30 minutes formed three layers. The center layer had a pea-like odor on warming.

Six ml. of the middle layer of the centrifuged mixture was dialyzed with Visking Type 8/100 tubing against 2 liters of distilled water. On being warmed, the dialysate did not smell like peas. This experiment demonstrated that the odor precursors are of low molecular weight.

The free amino acids were removed from reconstituted FDAE by passing the solution through a Dowex-50 ion exchange column. The FDAE was analyzed for diketo-L-gulonic acid, dehydroascorbic acid, and L-ascorbic acid by essentially the procedure developed by Roe et al. (19). The components identified in the FDAE are listed below.

Components of freeze-dried aqueous extract of frozen peas (FDAE) -- concentrations in mg./100 g. powder

Phosphatidyl inositol	Valine, 370
Phosphoryl glycerol	Leucine, 90
Phosphotidyl choline	Methionine, 30
Phosphotidyl ethanolamine	Threonine, 1000
or serine	Lysine, 440
Sucrose	Tyrosine, 70
Chlorophyll	α -Amino butyric acid, 50
Serine, 300	Canavanine, 200
Asparagine, 120	Homoserine, 320
Proline, 120	Phenylalanine, 70
Cystine, 20	L-Ascorbic acid, 183
Isoleucine, 180	L-Dehydroascorbic acid, 14
Glutamic acid	Diketo-L-gulonic acid, 6
Arginine, 3500	Histidine, 120
Glutamine, 1450	Glycine, 70
Alanine, 1700	

Preparation of Samples

The amino acids were the highest grade commercial samples. The L-isomer was used except in the case of D,L-methionine. D,L-methionine methiodide was synthesized and purified by the method of Toennies (23). The pectin used was the NF high methoxy product supplied by the Sunkist Growers Exchange, Lemon Products Division.

Canned peas containing additives were prepared as follows: clean, dry, C-enamel 303 x 406 cans were filled with 275 g. of commercially frozen Dark Skin Perfection peas. A 196-ml. portion of canning brine was added. The brine contained 12 pounds of salt and 24 pounds of sucrose per 100 gallons of water. The cans were vacuum closed at 16 to 22 inches of vacuum and were retorted in 3 basket loads of 40 cans each lying on their sides in a stationary retort. Cans came up to 100°C. in 5-10 minutes, were vented 3 minutes at that temperature, and cooked 30 minutes at 118°C. The cans were cooled with cold tap water in the retort for 20 minutes. The cooled cans were dried with a compressed air stream and by toweling and were stored in a cool (7° to 16°C.) warehouse until panel evaluation.

The additive samples were charged directly into dry cans by weighing solids or delivering liquids through micro syringes. All other conditions were identical to those of the control samples. Taste comparisons were made on cans from the same retort, charge, and storage conditions.

Taste Panel Evaluation

The first step in evaluating FDAE was to determine whether a large untrained panel would deem the odor of this material similar to the odor of peas in any form. FDAE has the interesting property of developing an odor when simmered in water. The odor from canned frozen peas was compared with that from FDAE reconstituted in distilled water heated to 70° to 80°C. Both samples were placed in polyethylene squeeze bottles and 40 untrained subjects were asked to rate the similarity of the odor from each sample against a list of 12 descriptive phrases. The response is tabulated in table 8.

About the same number of people ascribed pea odor to the FDAE as to the canned peas. This suggests that the odor from the reconstituted FDAE was very similar to that of canned peas.

We decided to determine whether the untrained panel could detect an odor difference between the reconstituted powder and pureed canned peas. This test showed the two samples were different (33 out of 41 judges correctly matched the duplicates) and,

furthermore, 35 out of 41 untrained judges preferred the odor from the pureed canned peas. FDAE smells like peas, but its smell is not as attractive as that of canned peas.

TABLE 8.--Number of times that FDAE and canned frozen peas were characterized by given descriptive phrases

Odor is most similar to that of:	Sample tested	
	FDAE	Canned peas
1. Fresh peas	12	10
2. Canned peas	24	26
3. Edible pod peas	4	8
4. Frozen peas	7	7
5. Split pea soup	12	10
6. Cooked Lima beans	11	5
7. Cooked Brussels sprouts	6	6
8. Cooked broccoli	4	5
9. Canned string beans	2	3
10. Canned spinach	4	1
11. Vegetable soup	1	2
12. Cooked mustard greens	4	4

We next compared canned peas with the same peas with varying amounts of FDAE added (table 9)..

TABLE 9.--Duo-trio comparison of commercial canned peas with and without added freeze-dried aqueous extract of frozen peas

Comparison	Percent correct matchings	Percent that preferred control
Control vs. 1 percent FDAE	66 (33/50)*	31*
Control vs. 1.5 percent FDAE	58 (28/48)	64
Control vs. 2 percent FDAE	81 (30/48)	67

*Significant at P = 0.05.

The results of the test of FDAE-modified canned peas were not promising. However, we decided to test portions of the complex mixture represented by FDAE.

The abundance of free amino acids and ascorbic acid in FDAE suggested that they might react to produce flavor components. Three beakers were each charged with 100 ml. of pH 6.0 phosphate buffer. One beaker was charged with 20 mg. of solid ascorbic acid, a second with 50 mg. of crystalline amino acid, and a third with both solids. Of the twelve amino acids tested, only the combination of methionine or phenylalanine with ascorbic acid produced significant odors (table 10). Phenylalanine gave a floral odor, methionine a cooked vegetable odor.

Dimethyl sulfide was a major component of the vapor above peas being blanched. The model experiments on amino acids and ascorbic acid suggested that one method of generating dimethyl sulfide in canned peas is by adding a combination of methionine with ascorbic acid. Recent work by Casey et al. (4) demonstrated that the combination of methionine and pectin produces dimethyl sulfide on heating. Table 11 summarizes the taste panel's reaction to canned peas containing additives that form dimethyl sulfide.

TABLE 10.--Results of a triangle comparison¹ of canned peas packed with and without amino acids as additives

Amino acid or peptide used	Level	Correct matching by 30 judges
	P.p.m.	Number
Arginine	2,940	14
Glutamine	20	16*
Homoserine	400	8
Alanine	945	12
Threonine	200	14
Cysteine	50	8
Glutathione	50	15
Methionine	470	23***
Phenylalanine	200	23***

*Significant at P = 0.05.

***Significant at P = 0.001.

¹Randomized order of presentation of one additive with two controls or one control with two additives.

TABLE 11.--Triangle test on canned pea samples containing additives

Additive	Level	Correct judgments	Judged better than control
	P.p.m.		
Methionine	470	23/30***	11/23
Ascorbic acid	200	22/29***	7/22
Methionine +	200	11/29	7/11
ascorbic acid	200		
Methionine +	100	16/30*	¹ 9/14
pectin	200		
Methionine + methiodide	5	17/30**	¹ 6/15
	50	19/30***	¹ 4/18*
Pectin	300	6/30	2/6
Methionine +	200	18/30**	6/18
pectin	300		

*Significant at P = 0.05.

**Significant at P = 0.01.

***Significant at P = 0.001.

¹One or two judges declined to express an opinion.

RELATED BASIC RESEARCH

Every research program reveals gaps in basic knowledge. Unprogrammed fundamental research is usually necessary to make the best progress on well defined programs.

In the present work on pea flavor we did ancillary research to fill in relevant areas of uncertainty. This work resulted in five publications (12, 15, 16, 17, 18). Because the detailed papers are readily available, only the background of the research and abstracts will be included in this report.

On heating frozen peas at 100°C. for 15 minutes the level of acetoin increased from 26 to 34 p.p.m; heating for 45 minutes lowered the concentration to 28 p.p.m. The increase of acetoin during the short heating is due to thiamine-catalyzed conversion of pyruvic acid to acetoin. This reaction was demonstrated with model systems in the pH range and reactant concentrations of canned vegetables (12).

In the determination of the trace nonvolatile organic acids in peas, a reaction used for preparation of ethyl pyrrolidone carboxylate gave a second product. The reaction of lactams with diazoalkanes in general was investigated to shed light on the unexpected observation. We found that five-, six-, and seven-membered lactams react sluggishly with diazomethane and diazoethane to produce O-alkyllactams or N-alkyllactams. Only traces of products form from lactams and diazoalkanes in ether solution. Alcohols promote the reaction. Caprolactam and valerolactam give O-alkyllactams with alcoholic-ethereal diazoalkane solutions. The N-alkylation of butyrolactam is catalyzed by fluoboric acid (15).

O-alkyllactams had been reported to undergo a thermal rearrangement to N-alkyllactams. We investigated this reaction with the help of a summer trainee (Carl A. Elliger). A sample of O-ethyl-caprolactim was highly purified by preparative GLPC. The compound was unchanged on heating at 193°C.; it produced trace amounts of N-ethylcaprolactam and moderate amounts of caprolactam during 21 hours' heating at $237^{\circ} \pm 5^{\circ}\text{C}$. The major thermal transformation of O-ethylcaprolactim at $285^{\circ} \pm 1^{\circ}\text{C}$. is first order ($k = 0.033 \text{ minute}^{-1}$) formation of caprolactim and ethylene. The ethylene was identified by GLPC retention time on an alumina column and by its mass spectrum. The reaction was 98 percent complete in 5 hours.

The rearrangement of O-alkyllactams to N-alkyllactams is effected by traces of dialkyl sulfate. A 1 mole-percent solution of diethyl sulfate in purified O-ethylcaprolactim was heated at $280^{\circ} \pm 10^{\circ}\text{C}$. The reaction was essentially complete in 12 minutes

and produced N-ethylcaprolactam and caprolactam in a GLPC peak area ratio of 3:1. O-methylvalerolactim is unchanged by heating at 250°C. for 5 hours. Addition of 5 mole-percent of dimethyl sulfate to O-methylvalerolactim and subsequent heating at $172^{\circ} \pm 1^{\circ}\text{C.}$ for 15 minutes converts O-methylvalerolactim almost completely to N-methylvalerolactam.

A 10 mole-percent mixture of dimethyl sulfate in O-ethylcaprolactim was heated for 25 minutes at 180°-190°C. The products of this reaction were diethyl sulfate, and N-methylcaprolactam. These results support the mechanism proposed for the transformation of O-methylcaprolactim to N-methylcaprolactam in the presence of dimethyl sulfate (16).

A literature survey of thermal rearrangements of organic compounds disclosed conflicting reports on the thermal stability of 2-allyloxyquinoline. We reinvestigated this question and prepared authentic 1-allyl-2-quinolone by the Robinson-Perkin reaction (17). 2-Allyloxyquinoline does rearrange to 1-allyl-2-quinolone on careful heating at 250°-260°C. At higher temperatures 1-propenyl-2-quinolone and 1-methyl-2-quinolone also form. Infrared and nuclear magnetic resonance spectroscopy established these results and demonstrated that no competitive Claisen rearrangement to carbon takes place.

We investigated the question of catalysis of unimolecular reactions because it is important in the general quality decline of food on heat processing. The thermal rearrangements of allyl 3-allyloxy-2-butenates and crotyl 3-crotyloxy-2-butenates were shown to be first order, intramolecular reactions. The rate constants are $2.7 \times 10^{10} e^{-26,300/RT}$ and $5.3 \times 10^{11} e^{-28,000/RT}$ second⁻¹, respectively. The reactions have moderate rates at temperatures as low as 115°C. The delta S* values of -11.3 and -5.4 e.u. were consistent with a cyclic transition state. All of the probable products and crossed products of the rearrangement were prepared. No crossed products were detected by analysis of the products of a mixed rearrangement by GLPC and infrared spectroscopy. Heterogeneous catalysis by ammonium chloride accelerates the rearrangements a little. Homogeneous catalysis by ammonium chloride or its dissociation products was excluded as an explanation of the increased rate by a proton magnetic resonance study of the rearrangements (17).

INDUSTRIAL APPLICATION

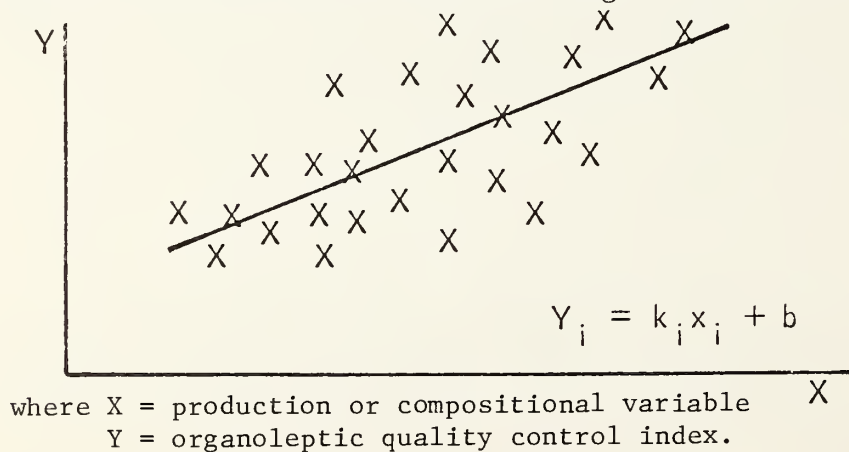
The industrially oriented food research worker will appreciate after reading this report that pea flavor is a complex mixture. Evidently we have only made a start on the problem of describing desirable processed pea flavor in terms of chemical

composition. The instability of certain pea components and the difficulty in establishing whether identified compounds are intrinsic components rather than artifacts of isolation hinder solution.

The chemical approach to a definition of pea flavor emphasized in this report has relegated the important variables of variety, agricultural practice, area of production, type of processing, etc., to secondary consideration. Correlation of all the factors influencing processed pea flavor would require a massive sample collection and analysis program.

The above considerations lead to the following recommendations: (1) that the basic research approach be interrupted, (2) that commercial pea processors integrate the information found in this report with their own unpublished findings to improve flavor acceptability of their future production, and (3) that the larger pea processors and combinations of the smaller processors (acting through regional or national associations) use quality control data to develop useful correlations that define desirable processed pea flavor.

The specific manner in which the last recommendation can be implemented will be described in detail. Using a large volume of data on the effect of a number of variables on the quality control rating of processed peas it is possible to derive a linear function which describes the most desirable product. The mathematical treatment of the data takes the following form:



Treatment of the flavor ranking of fresh (1.18), frozen (2.13) and canned (2.55) pea samples with the objective measurements on the same samples (table 6) gives a series of linear equations with widely different slopes. This variation exists because the amount of some components is not a true index of the

flavor of the product. Taste panels must choose which components will be useful objective measures of quality. The only compositional factors that taste panels have established as relevant are methionine and phenylalanine. A prerequisite for selection of data for further mathematical treatment is that they give a linear equation with a slope similar to that of an established correlating factor.

Data might also be interpreted through polyfunctional equations derived by computer analysis. Such treatment would correlate the principal peaks in the aromagrams (fig. 8) with quality rankings. Similarly, a relationship between organoleptic evaluation and amino acid composition could be developed. Further variables for analysis could be selected from variety, agricultural practice, location, processing, etc. This method of data treatment integrates the influence of a large number of variables on the flavor quality of the final product.

These methods of data treatment are within the scope of food industry research and quality control laboratories, and have been used on several fruit and vegetable products. For example, Scheltema, Sykes and Last (20) have correlated sensory quality of frozen peas, as assessed by preference ratings and acceptability percentages, with maturity and other factors. Linear regressions for the relationship between maturometer reading and AIS (alcohol-insoluble solids) were calculated. Schwimmer and Guadagni (21) have related the olfactory threshold and pyruvic acid content of onions. Boggs et al. (2) have measured by aromagrams the hexanal content of potato granules and used it as an index of deterioration. Heinz et al. (7) have correlated a hedonic index of the acceptability of pears with their content of 2,4-decadienoic acid. Vandercook et al. (24) have described a method for combining several compositional criteria to determine whether lemon juice meets certain standards of quality.

SUMMARY

Studies on the chemical composition of peas as related to flavor modification are reported. Among the nonvolatile components identified and determined were sugars, amino acids, polyfunctional organic acids, and lipids. The volatiles identified include aromatic hydrocarbons, terpenes, aldehydes, acetals, ketones, acids, alcohols, esters, and sulfur compounds. The method of flash exchange gas chromatography was developed and the new technique of combined gas chromatography and mass spectrometry was employed.

Fresh, frozen, and canned peas were evaluated for flavor quality and an attempt was made to correlate the results with differences in chemical composition. A reconstituted freeze-dried

aqueous extract (FDAE) of peas was found by a panel of judges to have an odor very similar to that of canned peas. However, neither FDAE nor other selected pea components showed promise as flavor-enhancing additives for use in canned peas.

Basic studies on (a) the conversion of pyruvic acid to acetoin; (b) reactions of lactams with diazoalkanes; (c) catalysis by dimethyl sulfate of the transformation of O-alkyllactams to N-alkyllactams; (d) thermal rearrangement of 2-allyloxyquinoline to 1-allyl-2-quinolone; and (e) kinetics and catalysis of the thermal rearrangement of alkenyl 3-alkenyloxy-2-butenates are briefly reviewed.

Finally, ways are suggested by which commercial pea processors might extend this research in order to arrive at useful correlations between objective knowledge of composition and organoleptically assessed pea flavor.

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APPENDIX

Isolation of Free Amino Acids from Freeze-Dried Aqueous Extract of Frozen Peas (FDAE) and from Frozen Peas

A 2.7-g. sample of the FDAE (93.5% solids) was deproteinized with 90 ml. of 1 percent picric acid in water and centrifuged at 16,000 x gravity for 20 minutes at 2°C. The supernatant was passed through a 1.3- x 24-cm. column of Dowex 2-10 percent ion exchange resin in the chloride form (50-100 mesh). The column was washed with 60 ml. of 0.02 N HCl. The eluants were combined and evaporated on a rotary film evaporator to about 3 ml. and made up to 10 ml. in a volumetric flask. A 3-ml. sample was taken and adjusted to pH 7.3 with 1 N NaOH and 0.4 ml. of 0.5 M Na₂SO₃ was added. After a 4-hour exposure to air the sample was adjusted to pH 2 with 1 N HCl and made up to 10 ml. with pH 2.2 sodium citrate buffer.

In a similar manner an 18-g. sample of frozen peas (21.1% solids) was blended with 90 ml. of 1 percent picric acid and treated by the procedure outlined above.

Hydrolyzed samples of FDAE and of frozen peas were prepared by treating 3 ml. aliquots of the concentrated eluants from the resin bed with 3 ml. of concentrated HCl in a tube suitable for vacuum sealing. The samples were frozen, evacuated, sealed under vacuum, and subjected to a 22-hour heating at 108°C. The tube was cooled, opened, and the contents filtered through sintered glass, washed, and evaporated to dryness with a rotary film evaporator and oil pump until all HCl was removed. These dried samples were made up in pH 2.2 citrate buffer. The samples run on the amino acid analyzer were diluted so that approximately 1 ml. (representing 25 mg. of peas, dry weight basis) was used per run.

Preparation of Volatile Fractions from 1960-Crop Peas and Preliminary Analysis by Gas Chromatography

Five thousand pounds of blanched frozen Dark Skin Perfection peas packed and shipped at -23.3°C. from Oregon in 1960 were processed in 400-pound lots. The peas were ground in a Fitzpatrick mill with an 1/8-inch screen. The mill was cooled before use by grinding and recycling 50 pounds of solid carbon dioxide. Grinding the peas took about 1 hour for each 400 pounds. The ground, frozen peas were then steam distilled in a 100-gallon, steam-jacketed, stainless steel kettle at 10 to 12 p.s.i.g.; an open

steam coil intermittently stirred the contents. The distillate was extracted immediately or was stored at -23.3°C . until extraction.

The volatiles were extracted from 70 gallons of pea steam distillate in 8-gallon portions with approximately equal quantities of purified isopentane in a Podbielniak liquid-liquid extractor. The solvent had been distilled through a 40-plate Oldershaw fractionating column at a 20:1 reflux ratio, b.p. 27.8°C .; it showed no other component than isopentane by gas chromatography and mass spectrometry. The 75 gallons of isopentane extract was dried over anhydrous sodium sulfate and concentrated by slow distillation at atmospheric pressure through a fractionating column until the head temperature rose above 27.8°C . The 700 ml. of pea extract concentrate was redried and concentrated to a small volume in smaller distillation equipment. Five fractions from the latter distillation were analyzed by mass spectrometry, and all were found to be pure isopentane. The concentrated pea extract consisted of 23.6 mg. of solid and 9.62 g. of liquid.

The extract was distilled at atmospheric pressure to yield 1.153 g. boiling at 24° to 29°C . (fraction 1). Further distillation at 65° to 100°C . and 15 torr gave 0.219 g. (fraction 2). Distillation at 28° to 100°C . and 0.4 torr provided 0.207 g. of liquid (fraction 3).

Fractions 1 to 3 were chromatographed on a commercial GLPC instrument (thermal conductivity detection) with a 10-foot x 1/4-inch column packed with 30 percent Apiezon "M" on 60- to 80-mesh washed Chromosorb to produce the chromatograms shown in figures 1 to 3. Tentative identification of fractions by GLPC were made on 1/8-inch x 5- to 10-foot stainless steel columns packed with 10 percent substrate (Carbowax 20 M and 1540, and Reoplex 400) on 60- to 80-mesh Chromosorb P.

Development of Procedure for Separating Volatile Components by Chemical Classes

Studies on model systems used the following compounds as a mixture: amyl amine, isovaleric acid, heptaldehyde, methyl isobutyl ketone, *n*-butyl alcohol, 3-butenenitrile, ethyl butyrate, and heptane. Isopentane was used as a solvent. The general procedure was to extract amines with 1 *N* H_2SO_4 and then acids with 5 percent NaHCO_3 . The isopentane was then evaporated, and the carbonyl compounds were allowed to react with ethanolic Girard's reagent T (trimethylaminoacetohydrazide chloride). The water-insoluble compounds were extracted with isopentane. The alcohols in the mixture

were separated by reaction with adipyl chloride; the unreacted acid chloride portion of the resultant ester was hydrolyzed. The half-ester was extracted with NaHCO_3 . Alternately, the ester, nitrile, and hydrocarbon components were removed by vacuum transfer. The free alcohols were regenerated by saponification of the adipic acid half-ester. Treatment of the ester-nitrile-hydrocarbon portion of the mixture with hot 25 percent NaOH solution gave acids from the nitriles, and acids and alcohols from the esters. This mixture was separated by a repetition of the earlier part of the separation method. The final fraction contained only the hydrocarbon. GLPC was employed to follow the course and efficiency of the separation at each stage.

The model mixtures were studied in 0.10 g. amounts dissolved in 2 to 3 ml. of isopentane. Free acids were extracted two times from the isopentane solution with 5 percent NaHCO_3 solution and either stored in the refrigerator or distilled after having been made strongly acid with 3 N H_2SO_4 . The steam distillate (500 ml.) was adjusted to pH 8.2 with 0.1 N KOH and evaporated on the steam bath to dryness. The potassium salts of the acids were determined as their ethyl esters by flash exchange gas chromatography for $\text{C}_1\text{-C}_6$ aliphatic acids.

The ketones and aldehydes were separated by first distilling off isopentane through vacuum-jacketed Vigreux columns. A solution of 0.15 g. of Girard's reagent T in ethanol was added to the distillation residue; this was followed by the addition of 50 mg. of Dowex 50 cationic resin (H^+ form) as a catalyst (9). The mixture was stirred for 3 hours at 25°C . with a Teflon-coated magnetic stirring bar. A condenser prevented losses by evaporation. The heptaldehyde reacts almost immediately, but methyl isobutyl ketone reacts slowly, and the reaction did not reach completion. The best conditions for the removal of carbonyl compounds were a 3-hour heating period at 80°C . The reaction mixture was cooled, water was added, and the mixture was washed with isopentane to remove alcohols, esters, nitriles, and hydrocarbons.

The aqueous extract containing the bound carbonyl compounds was acidified with 3 N H_2SO_4 and steam distilled. The 500 ml. of distillate was extracted for 5 hours with isopentane in a liquid-liquid extractor. The extract was concentrated to a small volume with a Rotovac evaporator.

The next class of compounds, the alcohols, must be separated from the esters, nitriles, and hydrocarbons. From the first model studies it was apparent that some loss of lower molecular weight alcohols was inevitable in the ethanolic Girard's reagent treatment. The problem of finding a good reaction to remove the alcohols from the rest of the mixture was solved only after several abortive approaches.

Mixtures of alcohols in heptane were treated with one mole of adipyl chloride for 1 to 2 hours at reflux temperature. The course of the reaction was followed by GLPC. We assumed that the alcohols would form mono-esters, but the results indicated that considerable diester formed. After hydrolysis of the reaction mixture with NaHCO_3 and separation of the aqueous portion, the heptane phase was concentrated, saponified with KOH , and found by GLPC to contain most of the alcohols. The aqueous portion, which was thought to contain the sodium salt of the monoester-monoacid, was hydrolyzed. GLPC analysis of the heptane extract of the mixture showed virtually no alcohols. Studies made after running the first fraction of the pea extract material showed that the higher alcohols reacted completely at 25°C ., but n-amyl alcohol and lower alcohols reacted only to the extent of 50 percent.

The next classes of compounds, esters and nitriles, represented by ethyl butyrate and 3-butenenitrile, were concentrated in isopentane solution and saponified with 25 percent NaOH . The cooled saponification mixture was washed with isopentane to remove alcohols and hydrocarbons. The aqueous portion was acidified with 3 N H_2SO_4 and steam distilled. The distillate was adjusted to pH 8.2 and evaporated. The resulting potassium salts were run as described before. Butyric acid was recovered in 90 percent yield from the saponification of ethyl butyrate. The nitrile did hydrolyze to crotonic acid, but its recovery was poor due to loss by volatilization in isopentane distillation and by solution in ethanol during the carbonyl separation. Hexanenitrile was also used as a model and successfully recovered as ethyl hexanoate. The alcohol portion of the ester was separated from the hydrocarbon by use of adipyl chloride. Ethanol from ethyl butyrate was obscured in chromatography, so an ester containing a higher alcohol was studied.

A mixture of equal volumes of isoamyl isovalerate, ethyl butyrate, and hexanenitrile (60 μl . total) in 1 ml. heptane was refluxed in alkaline diethyleneglycol for 2 hours at 200°C . A steam distillate from the alkaline solution was extracted with isopentane in a liquid-liquid extractor for 5 hours. The isopentane extract was distilled to a small volume, and GLPC showed a good recovery of isoamyl alcohol.

Analysis of Fraction 2 -- 1960 Peas

An analysis of a portion of fraction 2 showed N, 2.86 percent and S, 10.5 percent. An infrared spectrum run as a film between NaCl plates showed the following absorption peaks: 3,335, 2,950, 2,920, 2,860, 1,696, 1,665, 1,455, 1,376, 1,060, 888, 800, and 760 cm^{-1} . The remainder of the fraction was transferred to a 3 ml. glass-stoppered centrifuge tube with 1 ml. of heptane. The solution was extracted three times with 0.75 ml. of cold 3 N HCl

(first extract orange, last colorless). The HCl extracts were steam distilled, and the residue was evaporated to yield 9.5 mg. of solid.

The heptane extract was treated with adipyl chloride. After a 3-hour reaction time at 25°C., a vacuum transfer of the non-alcoholic portion was made at 75°C. and 15 torr. The residue was saponified with 30 percent KOH by heating in an oil bath at 80° to 135°C. under reflux with ice-water circulating through the condenser. After 3 hours, the mixture was cooled and extracted with four 0.8-ml. portions of heptane. The heptane extract was analyzed by GLPC on a Carbowax 1540 column at 110°C. Seven peaks appeared; the three largest were at 33.7, 40.1, and 53.6 minutes.

The heptane-transfer material from the removal of alcohols contained more than twenty peaks when analyzed by GLPC on Carbowax 20 M, Carbowax 1540, and Reoplex 400 at 80° to 145°C. The heptane solution had a low concentration of compounds; it was concentrated by making 50 μ l. injections into a 1/4-inch by 3-foot, 20 percent Carbowax 20-M column at 110°C. All material after the heptane peak was collected in a glass U-tube which was packed with glass helices and cooled in an acetone-solid carbon dioxide bath. Twelve collections were made. The material in the U-trap was vacuum transferred to a small pear-shaped test tube under high vacuum; a mercury diffusion pump was used. A total of 32 mg. of material was collected. The upper layer was about 10 mg. of concentrated heptane solution, and the lower layer was water. This material was analyzed by GLPC mass spectrometry.

Analysis of Fraction 3 -- 1960 Peas

The 0.207 g. of fraction 3 was transferred to a cold glass-stoppered test tube with 3 ml. of cold isopentane. The solution was washed four times with 2-ml. portions of NaHCO_3 to remove free acids. The aqueous extract was back extracted with two 1-ml. portions of isopentane. The NaHCO_3 extract was acidified and steam distilled. The 500 ml. of distillate had a small amount of suspended solid. The solid was extracted with ethyl ether; evaporation of the dried ethereal solution gave 5.9 mg. of solid. The infrared spectrum showed strong C-H stretching and C=O stretching, and the material appeared to be a mixture of C_{16} to C_{18} fatty acids. The filtrate was adjusted to pH 8.25 and washed with isopentane in a liquid-liquid extractor. The aqueous portion was evaporated to dryness to yield 10.5 mg. of potassium salts which were analyzed by flash exchange gas chromatography. The 250-ml. isopentane extract of the alkaline distillate was concentrated by distillation. GLPC of this residue on the above column showed eight peaks post-isopentane. These compounds, water-soluble components of the mixture, were not identified because of lack of material.

In the isopentane solution of the pea extract, a gummy yellow-red material was suspended. It was soluble in CCl_4 . Infrared spectroscopy showed only carbon-hydrogen stretching as a significant absorption. This material may be a polymeric product of one or more components of the pea extract.

The isopentane solution, after removal of the acids, was concentrated by distillation. The residue was treated with 0.3 g. trimethylaminoacetohydrazide chloride, 4 ml. of ethanol, 0.2 ml. of water, and 85 mg. of Dowex 50 resin. The mixture was heated for 4 hours at 70°C . bath temperature (odor of H_2S detected). The cooled mixture was extracted with four 4-ml. portions of isopentane. The aqueous layer was rinsed into a distillation flask with water, acidified with 3 N H_2SO_4 , and steam distilled. The 500 ml. of distillate collected was extracted with isopentane in a liquid-liquid extractor. The extract was concentrated to a volume of 0.4 ml. by distillation. GLPC, on a 20 percent Carbowax 1540 column at 91°C ., revealed eleven peaks. One was tentatively identified as isovaleraldehyde. The largest peak at 12.4 minutes gave a negative test for carbonyl with 0.2 percent 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. The early peaks were not identified, and the later peaks, if they were aldehydes or ketones, were above $\text{C}_9\text{-C}_{10}$.

The isopentane extract from the carbonyl separation was concentrated by distillation. The residue was treated with 0.45 ml. of adipyl chloride and the mixture kept at 25°C . for 3 hours. HCl evolved. The reaction flask was put on a Rotovac unit and volatile material transferred to a trap cooled in ethanol-solid carbon dioxide at 20 torr and then at 1 torr pressure. Three portions of heptane were added to the reaction flask and the solvent vacuum transferred. A white, waxy solid was present in the collection trap and glass connections. The solid had an odor reminiscent of peas. The material was insoluble in water and had a melting point of 147.5° to 149°C . compared to 150° to 151°C . for odorless adipic acid. The infrared spectrum was identical to that of adipic acid. Apparently, the pea odor was due to traces of material absorbed on the surface of the adipic acid.

The esterified alcohol fraction was saponified with 3.5 ml. of 30 percent KOH for 3 hours at reflux temperature. The cooled reaction mixture was extracted 2 times with 1-ml. portions of heptane. The heptane solution was analyzed by GLPC on a Carbowax 20 M column at 142°C . Tentative identifications were made from a plot of log retention time vs. carbon content for known alcohols. The three largest peaks were assigned the structures of n-C_7 , secondary C_{10} , and secondary C_{11} or iso-C_{10} .

The vacuum-transferred material in heptane from the adipyl chloride reaction was saponified with a mixture of 0.4 ml. water, 0.6 g. KOH, and 1.8 ml. diethyleneglycol in an oil bath at 195° to 215°C. for 2 hours. The solution turned dark on heating and gave off an "oil refinery" odor. Extensive decomposition took place; the formation of black particles (probably carbon) was observed. The heptane layer was separated from the cooled reaction mixture. The aqueous layer was acidified and steam distilled. During the steam distillation an odor like H₂S came off, and a colorless solid material was suspended in the distillate. The pH of the distillate was adjusted to 8.2 and it was evaporated to yield 36 mg. of potassium salts. A mixture of 25 mg. of the salts with 25 mg. of potassium ethyl sulfate was heated for 5 minutes at 280° to 300°C. The volatile reaction products were collected in a flask cooled in ethanol-solid carbon dioxide. GLPC of this material on diethyleneglycol succinate at 153°C. showed 4 peaks. The heptane layer from the saponification was treated with 0.3 ml. of adipyl chloride and stirred for 4 hours at 25°C. A vacuum transfer at 15 torr was made with 1.2 ml. of heptane as a carrier. The residue after the transfer was saponified with 2 ml. of 30 percent KOH at an oil bath temperature of 160°C. for 15 minutes, then 135°C. for 3 hours. The mixture was cooled, diluted with water, and extracted with heptane. Tentative identification of the alcohols was made by GLPC on three different columns.

Artifacts of the Isolation Procedure -- 1960 Peas

The residue (9.07 g.) from the distillation consisted of two layers. The lower phase was dark brown and the upper phase was light yellow and quite viscous. The latter (1.05 g.) was separated from the bottom layer in a separatory funnel. The infrared spectrum showed it to be largely silicone lubricant which had been used on the extractor tanks.

The bottom phase showed strong carbon-hydrogen, ester carbonyl, and ortho-substituted benzene absorption in the infrared. The 2,4-dinitrophenylhydrazine test for carbonyl groups was negative (although there was a slight turbidity). A 0.5-g. portion of the bottom phase (total weight 8.01 g.) was saponified 4 hours with aqueous NaOH at 110° to 130°C. bath temperature. Extraction with ether, drying the ethereal solution over anhydrous sodium sulfate, and evaporation gave 0.5 ml. of liquid consisting of two phases.

GLPC of the upper phase showed 11 peaks; the largest, at 55 to 65 minutes was very broad. The alkaline portion was acidified with H₃PO₄. The odor gave no evidence of C₂-C₅ aliphatic acids. After standing overnight, the gelatinous suspension was

extracted with ether. Evaporation of the ethereal solution gave a trace of reddish oil in water. The oil-water mixture was dissolved in benzene and distilled to remove water. The acidic material was esterified in methanol and p-toluenesulfonic acid. GLPC showed little or no material in the higher boiling aliphatic ester range. The saponification was repeated with 1.5 g. of the material. 0.9 g. of red oil was extracted from the saponification mixture with benzene. The oil was purified by preparative GLPC to give 0.5 ml. of the major component. Mass spectrometry and proton nuclear magnetic resonance showed this material to be 2-ethyl-1-hexanol. Calculated for $C_8H_{18}O$: C, 73.8; H, 13.9. Found: C, 73.2; H, 13.8.

A Soxhlet extraction of the Tygon tubing used to make some transfers of the isopentane extract gave 36.1 g. of residue from 100 g. of tubing. The infrared spectrum of the material was identical to that of the bottom layer residue described above. The major portion of the pea extract distillation residue therefore was the plasticizer 2-ethylhexyl phthalate. A solid in the aqueous layer from the saponification was filtered off and the filtrate acidified with H_3PO_4 to pH 2 (sulfurous odor). A brown precipitate formed. The steam distillate contained a suspended yellow solid. A solid whose melting point and mixed melting point were the same as sublimed sulfur was extracted with carbon disulfide.

Control Analyses and Tests -- 1962 Peas

A 300-g. portion of the unexposed charcoal was extracted for 8 hours with 500 ml. of purified dichloromethane. The extract was concentrated to 0.5 ml. by distillation. The material was still primarily dichloromethane as shown by GLPC. The extract was further concentrated to 0.072 g. This material was analyzed by the Cap-MS method, and the mass 43 recording is reproduced in figure 11.

Two liters of the original blancher condensate was extracted continuously with ethyl ether in a liquid-liquid extractor for 8 hours. The extract was dried and concentrated. Analysis by GLPC showed no peak corresponding to DMSO. Similar leaching of dilute aqueous solutions of DMSO with ether resulted in extract concentrates that on GLPC analysis gave a peak corresponding to DMSO.

The vapor above a dilute solution of dimethyl sulfide in water was drawn through a U-tube filled with fresh charcoal for 8 hours. The charcoal was freeze-dried and extracted with dichloromethane. The extract was concentrated and analyzed by GLPC; a peak was found that had the same retention time as authentic DMSO. On the basis of these observations, we conclude that the

DMSO found in the lower phase of the concentrated extract was an artifact of isolation.

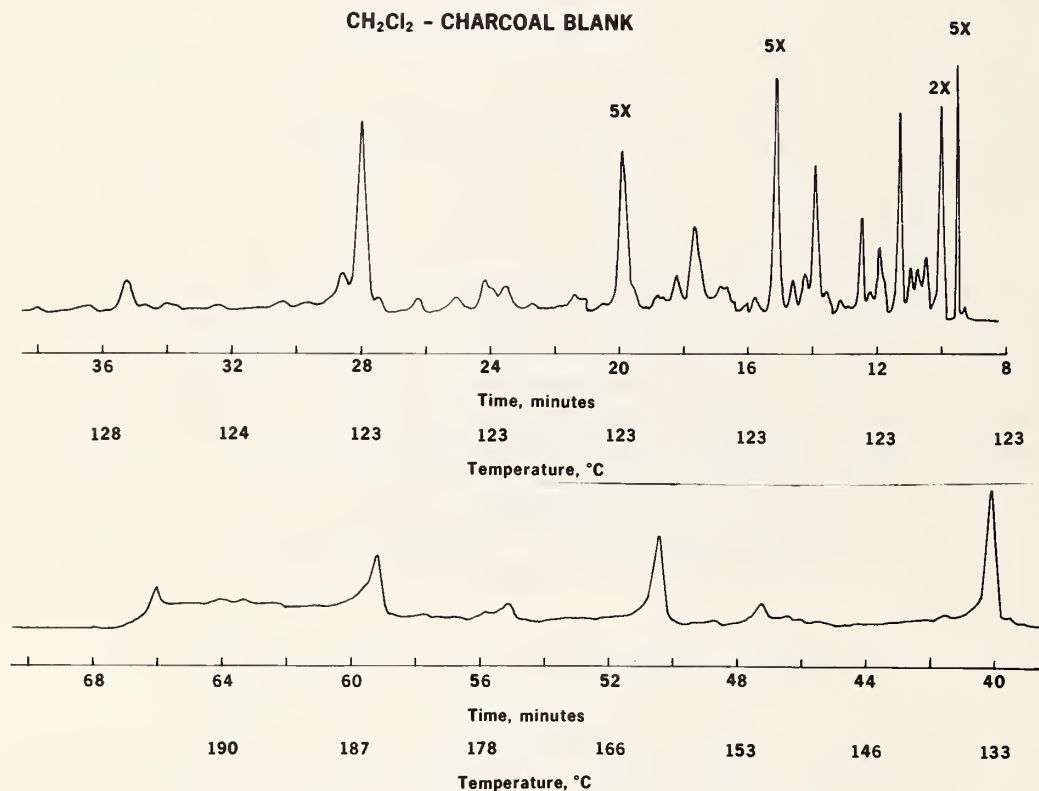


Figure 11.--Gas chromatogram of charcoal extract.

An aqueous solution of 5 g. of ethanol and 0.120 g. of isovaleraldehyde in 250 ml. of water was placed in a 500-ml. flask, and the vapor above the solution pulled through charcoal filled traps. The collection of vapor continued over 16 hours with two more additions of ethanol and isovaleraldehyde to the flask. The 336 g. of charcoal increased in weight to 363 g. The charcoal was freeze-dried and extracted with 500 ml. of purified dichloromethane. The extract (400 ml.) was dried with anhydrous sodium sulfate and concentrated by distillation to a volume of 10 ml. The concentrate was further concentrated in a rotary evaporator to a volume of 0.5 ml. The concentrate was analyzed on the Dow-Corning 710 column. A peak corresponded in retention time to 1,1-diethoxy-3-methylbutane, but it was no larger in area than two adjacent peaks. Analysis of the concentrate by Cap-MS established the presence of 1,1-diethoxy-3-methylbutane and isovaleraldehyde.

Preparation of Freeze-Dried Aqueous Extract (FDAE)

Dark Skin Perfection peas were obtained from a commercial freezer in Oregon in 1963. The peas came from fields that yielded approximately 6,000 pounds per acre at 109 to 111 tenderometer reading. The peas were cut in the field and then hauled 15 miles to a vining station. After vining, the peas were cooled in cold water, dewatered, and loaded into tank trucks. The time lapse between vining and processing was 2 to 3.25 hours. The peas were air cleaned, washed, run through a froth flotation cleaner, and then water blanched for 90 seconds at 96°C. After water cooling, the brine separator gave a fancy fraction which was inspected, frozen, and packaged.

About 878 g. of the freeze-dried extract material was prepared from 13.1 kg. of frozen peas. Portions of 550 g. of peas were blended with 550 ml. of distilled water for 2.5 minutes, transferred to cold stainless steel centrifuge cups, and centrifuged 15 minutes at 16,000 x g. at 2°C. The liquid was decanted and recentrifuged. Two hundred ml. of cold water was used in the transfer. The extract was poured into stainless steel trays in a -29°C. cold room. The frozen extract samples were transferred within a period of 5 minutes to a Stokes vacuum drier and dried at 0.4 torr, 27°C. plate temperature for 25 hours. The total yield of freeze-dried aqueous extract (FDAE) was charged into polyethylene bags in a 2 percent relative humidity room. The bags were placed in a can containing CaCl_2 desiccant and stored at -29°C. until use.

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